Medical and Pediatric Oncology 28:325–332 (1997)

Detection of Chimeric mRNAs by Reverse Transcriptase-Polymerase Chain Reaction for Diagnosis and Monitoring of Acute Leukemias With 11q23 Abnormalities

Kohmei Ida,[1] Tomohiko Taki,[1] Fumio Bessho,[1] Miyuki Kobayashi,[1] Fumiko Taira,[2] Ryoji Hanada,[3] Keiko Yamamoto,[3] Yuri Okimoto,[4] Masao Seto,[5] Ryuzo Ueda,[6] and Yasuhide Hayashi[1]*

Recurrent translocations involving chromosome band 11q23 are often found in human acute leukemias. Recently, the *MLL* gene on 11q23 and 10 partner genes involved in these translocations have been cloned and characterized. We performed a reverse transcriptase-polymerase chain reaction (RT-PCR) to detect the resultant der(11) chimeric mRNAs of the 3 types of 11q23 translocations including t(4;11), t(9;11), or t(11;19), in 14 leukemia patients with *MLL* gene rearrangements. At diagnosis or relapse, chimeric mRNA could be detected in all of the 4 patients with t(4;11), 2 of 3 with t(9;11), 2 of 3 with t(11;19), and 1 of 4 with unsuccessful karyotype. In 5 patients, we could monitor minimal residual disease (MRD) serially through the clinical course. One patient, in whom chi-meric mRNA was detected during complete remission (CR) just after the induction chemotherapy, relapsed within 2 months and died, while 2 patients in which chimeric mRNA was not detected remained in CR from 10–23 months. These findings suggest that RT-PCR is a useful approach for detecting which partner gene is involved in the translocation and monitoring MRD in patients with *MLL* gene rearrangement. Nonetheless, the clinical relevance of MRD evaluation by RT-PCR monitoring remains controversial. Long-term and prospective investigation of a larger series of patients is needed to confirm the clinical significance of monitoring MRD by RT-PCR method. Med. Pediatr. Oncol. 28:325–332, 1997.

Key words: acute leukemia; 11q23 abnormalities; MLL gene; RT-PCR; minimal residual disease

INTRODUCTION

Recurrent translocations involving chromosome band 11q23 are often found in human acute leukemias [1], especially infant acute leukemias [2–5] and therapy-related acute myelogenous leukemias (t-AML) [6–10]. Recently, a gene designated *MLL* [11,12], *HRX* [13], *ALL-1* [14,15], or *HTRX1* [16] has been cloned and shown to be disrupted by these translocations. *MLL* gene encodes for a predicted 431-kD protein, a human homolog of the *Drosophila* trithorax protein [13,14], which contains two putative DNA-binding motifs consisting of multiple zinc fingers located centrally and several AT hooks at N-terminus, and also has a DNA methyltransferase domain positioned upstream of the zinc fingers [13,14,17,18]. Recently, *MLL* protein has been shown to act as a transcriptional factor [19].

Genomic breakpoints on *MLL* gene cluster in a restricted 8.3-kb portion between exons 5 and 11, and 0.9-kb *Bam*HI fragment cDNA probe can detect *MLL* gene rearrangement in almost all of the cases [20]. To date, 10 partner genes in these reciprocal translocations have been cloned and characterized. They were designated *AF-1p* on 1p32 [21], *AF-1q* on 1q23 [22], *AF-4/LTG4/FEL* on 4q21 [14,23,24], *AF-6* on 6q27 [25], *AF-9/LTG9* on 9p22 [24,26], *AF-10* on 10p12 [27], *AF-17* on 17q21 [28], *ENL/LTG19* on 19p13.3 [24,29], *ELL/MEN* on 19p13.1 [30,31], and *AF-X1* on Xq13 [32], respectively. Moreover, it was reported that *MLL* gene was rearranged through partial tandem duplication without any reciprocal translocations in patients with AML [33,34]. It is predicted that alteration of normal *MLL* protein transcriptional function by the cleavage of *MLL* protein or by the production of various fusion proteins is responsible for leukemogenesis.

Clinically, leukemias with 11q23 translocations account for approximately 50 percent of AMLs and acute

[1]Department of Pediatrics, Faculty of Medicine, University of Tokyo, Tokyo, Japan; [2]Mitsubishi Kagaku Bio-Clinical Laboratories, Tokyo, Japan; [3]Division of Hematology/Oncology, Saitama Children's Medical Center, Saitama, Japan; [4]Division of Hematology/Oncology, Chiba Children's Hospital, Chiba, Japan; [5]Laboratory of Chemotherapy, Aichi Cancer Center Research Institute, Nagoya, Japan; [6]Department of Internal Medicine II, Nagoya City Medical School, Nagoya, Japan.

*Correspondence to: Yasuhide Hayashi, Department of Pediatrics, Faculty of Medicine, University of Tokyo, Hongo 7–3–1, Bunkyo-ku, Tokyo 113, Japan.

Received 11 April 1996; Accepted 28 June 1996

TABLE I. Clinical, Cytogenetic, and RT-PCR Data on 14 Patients

Patient no.	Sex/age	Karyotype	FAB	RT-PCR results
1	F/4 mo	46, XX, t (4;11)	L2	MLL/AF-4
2	M/2 mo	46, XY, t (4;11)	L2	MLL/AF-4
3	M/9 mo	46, XY, t (4;11)	L2	MLL/AF-4
4	M/15 yr	46, XY, t (4;11)	L2	MLL/AF-4
5	M/10 yr	46, XY, t (9;11)	M5a	MLL/AF-9
6	F/10 mo	46, XX, t (9;11)	M5a	MLL/AF-9
7	F/0 mo	46, XX, t (9;11)	M5a	—
8	M/7 mo	46, XY, t (11;19)	L2	MLL/ENL
9	F/3 mo	46, XX, t (11;19)	L2	MLL/ENL
10	F/3 mo	46, XX, t (11;19)	L1	—
11	F/2 mo	nd	M5a	MLL/AF-9
12	M/10 mo	nd	L1	—
13	F/10 mo	nd	L2	—
14	M/8 yr	nd	L2	—

nd: not determined.

lymphoblastic leukemias (ALLs) in infants, with the t(4;11), t(9;11), and t(11;19) being the most common [2–4,35]. Infant acute leukemias with 11q23 translocation often present aggressive clinical features and several investigators have found that infant ALLs with *MLL* gene rearrangement show a much poorer prognosis than those without *MLL* gene rearrangement [36,37]. Therefore, a rapid and accurate method for diagnosing these types of leukemia and monitoring minimal residual disease (MRD) is preferable in selecting a therapeutic approach.

In this study, we performed a reverse transcriptase-polymerase chain reaction (RT-PCR) to detect the resultant chimeric mRNAs of 3 types of 11q23 translocations including t(4;11), t(9;11), or t(11;19), in 14 leukemia patients with *MLL* gene rearrangements. Chimeric mRNAs could be detected in 9 patients, including one with unknown karyotype. In 5 patients, we could monitor the MRD serially through the clinical course. These findings suggest that RT-PCR is a useful approach for detecting which partner gene is involved in the translocation and monitoring MRD in patients with *MLL* gene rearrangement whose fusion message could be PCR amplified at diagnosis.

MATERIAL AND METHODS

Patients and Samples

Fourteen patients with de novo acute leukemia were examined. We selected these patients on the basis of *MLL* gene rearrangement on Southern blot analysis after screening of 31 childhood acute leukemias. Their ages ranged from 0 day to 15 years (Table I). ALL and AML patients were treated according to the Tokyo Children's Cancer Study Group (TCCSG) L84–11 or L89–12 protocols [38] and the 12th-ANLL protocol [39], respectively. Diagnosis was established based on standard French-American-British (FAB) morphologic and cytochemical criteria [40]. The percentage of leukemic cells of the samples were more than 80%. Mononuclear cells were isolated from bone marrow (BM) or peripheral blood (PB) samples collected at diagnosis by Ficoll-Hypaque centrifugation. We were able to obtain several samples from 5 patients through the clinical course (Fig. 4). Chromosomal analysis was performed by the routine trypsin-Giemsa- or Q-banding method [41], and immunophenotyping of the leukemic cells was performed as previously described [42].

Southern Blot Analysis

Southern blot analysis was conducted as previously described [42,43]. All DNAs were digested with *Bam*HI endonuclease, and in some of the 14 patients they were also digested with additional endonuclease such as *Xba*I, *Bgl*II, and *Eco*RI. Ten μg of digested DNAs were electrophoresed in TAE buffer (40 mM Tris, 20 mM sodium acetate, 2 mM EDTA pH 7.2) through 1.0% agarose gel (FMC Bioproduct, Rockland, ME, USA) at 28 V overnight, transferred to a nylon filter (Biodyne B, PALL, Glen Cove, NY, USA) and fixed to the filter by ultraviolet light (Stratalinker, Stratagene, La Jolla, CA, USA). Hybridization was performed in 50% dextran sulfate solution for 48 hours with Probe A or Probe B ^{32}P-labeled by random priming. Probe A is an approximately 0.9 kb-length *Bam*HI fragment of MLL cDNA (corresponding to the probe x in reference 29), whereas Probe B is a genomic probe produced by the PCR-amplification between exons 7 and 8. The locations of these 2 probes are shown in Fig. 1. The filter was then washed three times with 0.2 × standard saline citrate (SSC) containing 1% sodium dodecyl sulfate at 55°C for 20 minutes, and autoradiography was performed with X-ray film at −80°C for 2–4 days.

RNA Preparation and RT-PCR Analysis

Total cellular RNA was obtained by acid guanidine thiocyanate-phenol-chloroform extraction as previously reported [44]. Four μg of total RNA were reverse transcribed in a volume of 20 μl containing 2.5 U of Molony murine leukemia virus reverse transcriptase, 50 pmol random primers, 1 mmol/L dNTP, and 20 U of RNase Inhibitor using a commercial kit (Amarsham, Buckinghamshire, England) for 60 minutes at 42°C. For amplification of the cDNA products, 100 μl of PCR mixture containing 1 mmol/L $MgCl_2$, 50 mmol/L KCl, 10 mmol/L Tris-HCl, pH 8.3, 100 μmol/L dNTP, 2.5 U of Taq DNA Polymerase (Perkin Elmer-Cetus, USA), and 25 pmol single pair of primers selected according to the result of chromosomal analysis were added, and samples were overlaid with mineral oil (Sigma, St Louis, MO, USA). We performed after an initial denaturation step of 2 minutes at 94°C, 35 cycles consisting of 2 minutes at

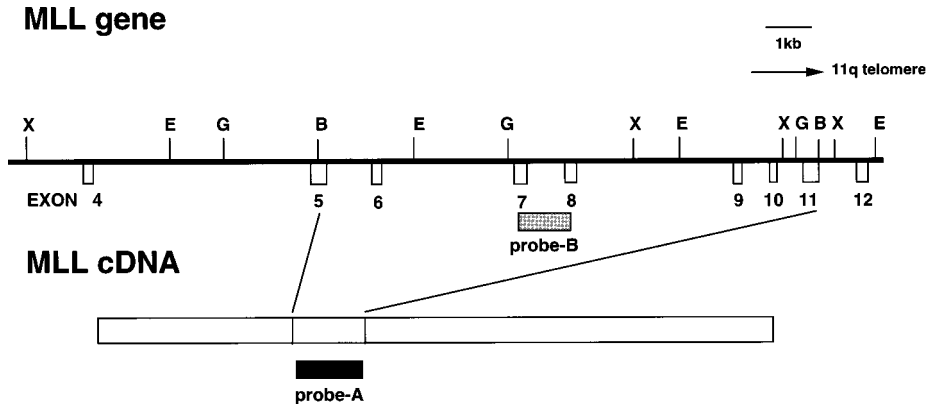

Fig. 1. Partial physical map of *MLL* gene and cDNA with the locations of probe A and probe B used for this study. Restriction sites are indicated by capital letters: B, *Bam*HI; E, *Eco*RI; G, *Bgl*II; X, *Xba*I. Open boxes with numbers denote the exons of *MLL* gene.

94°C, 2 minutes at 62°C, and 2 minutes at 72°C followed by a final extension of 4 minutes at 72°C were performed. The amplified products were electrophoresed on 3.0% agarose gel, stained with ethidium bromide and viewed under a UV lamp. To assess the sensitivity of RT-PCR amplification in this study, RNA obtained at diagnosis or relapse in patients 1, 2, 4, 6, and 9 was serially diluted by mixing with normal PB, reverse transcribed, and amplified, respectively. Primers used in this study were as follows: MLL-EX6 (exon 6 of *MLL* gene), 5′-GCAAACAGAAAAAAGTGGCTCCCCG-3′; MLL-EX7 (exon 7 of *MLL* gene), 5′-GAGGATCCTGCCCCAAAGAAAAG-3′; LTG4-AS (*AF-4/FEL/LTG4* gene), 5′-TGAGCTGAAGGTCGTCTTCGAGC-3′; LTG9-AS (*AF-9/LTG9* gene), 5′-CTGTGAAGCTCTACCAGTTC-3′; LTG19-AS1 (*ENL/LTG19* gene), 5′-AGCGTACCCCGACTCCTCTACTTTTG-3′; LTG19-AS2 (*ENL/LTG19* gene), 5′-GACGAAGAGTCGTCCTCGTCGGACT-3′. For all samples, amplification of β-actin cDNA was performed as an internal control. Normal PB was used simultaneously as a negative control at every step in both the RNA extraction and PCR experiment (data not shown).

RESULTS

The results of cytogenetic analysis and RT-PCR were shown in Table I. Ten of 14 patients were successfully karyotyped. All the patients with t(4;11) and t(11;19) were diagnosed as having ALL-L1 or -L2, and those with t(9;11) as AMOL-M5a. Thus, there was a good correlation between the type of translocation and the phenotype as reported previously [45]. In immunophenotyping, all of the leukemic cells from the patients diagnosed as having ALL-L1 or L2 expressed CD19 and HLA-DR antigens, but not CD10 antigen, whereas those from the patients diagnosed as M5a expressed one or more myeloid antigens, but not CD19 or CD10 antigen.

As shown in Fig. 2, Southern blot analysis of these patients revealed that the majority of breakpoints clustered in the restricted region between exons 5 and 11. Only patient 7 had an exceptional breakpoint, which was located in the 3′ region of exon 11. The distribution of the breakpoints did not differ among 3 types of translocation. In patients 4 and 11, we could not identify the breakpoint site because we used only a single restriction enzyme (*Bam*HI) and a single probe (probe A). The breakpoints on *MLL* gene fall in the 3′ region of exon 6 according to these results. Therefore, we set a sense primer in exons 6 and 7 in order to cover the most 5′ breakpoints. For the partner genes, we set antisense primers downstream of the breakpoints which were reported by us [26,29] and another group previously [23].

Chimeric mRNAs could be detected at diagnosis or relapse in 9 to 14 (64%) patients by RT-PCR method in this study (Table I). We could detect chimeric mRNAs in all of the 4 patients with t(4;11) when a set of MLL-EX7 and LTG4-AS primers was used, but it was not detected when a set of MLL-EX6 and LTG4-AS primers was used. We could detect chimeric mRNAs in 2 of 3 patients each with t(9;11) and t(11;19) using a set of primers shown in MATERIAL AND METHODS. Furthermore, *MLL-AF9/LTG9* fusion gene could be detected in one of 4 patients with unknown karyotype.

In 5 patients (1, 2, 4, 6, and 9), we performed RT-PCR analysis of serial samples from PB and BM through the clinical course to evaluate the usefulness of this approach for the detection of MRD. We could not detect the chimeric mRNA in any BM or PB samples from patients 4 and 6 after they had achieved complete remission (Figs. 3, 4), suggesting leukemic cells had been eradicated at the molecular level. However, our treatment failed to induce a molecular clearing of the leukemic cells in patients 1, 2, and 9. Patient 2, in whom the chimeric mRNA could be detected even in hematologic CR phase, relapsed within 2 months after the chemotherapy and died (Figs. 3, 4).

In the sensitivity testing of these 5 patients, the PCR

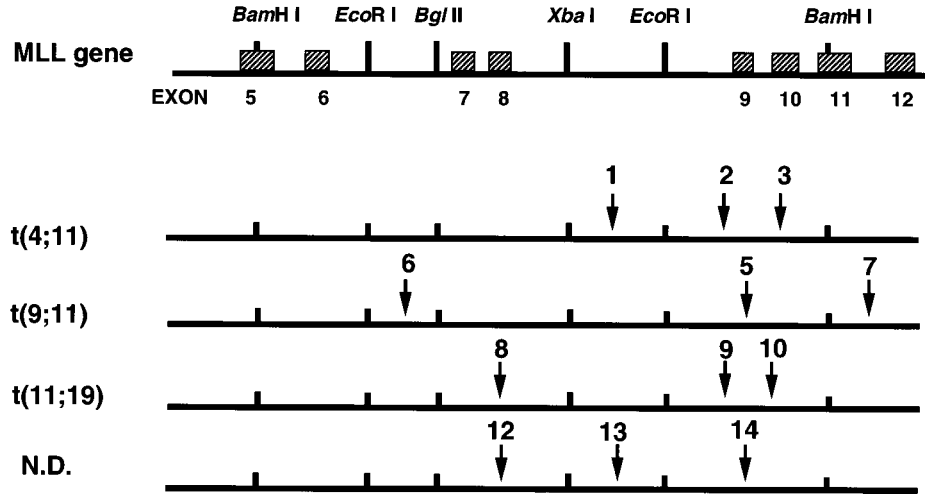

Fig. 2. Putative breakpoints on partial physical map of *MLL* gene. The breakpoint positions estimated by Southern blot analysis are indicated by vertical arrows separately according to the type of *MLL* gene translocation. The number on the arrow refers to the patient number indicated in Table 1. Restriction sites are indicated by vertical solid lines. Exons of *MLL* gene are represented by hatched boxes with exon numbers. N.D., not determined.

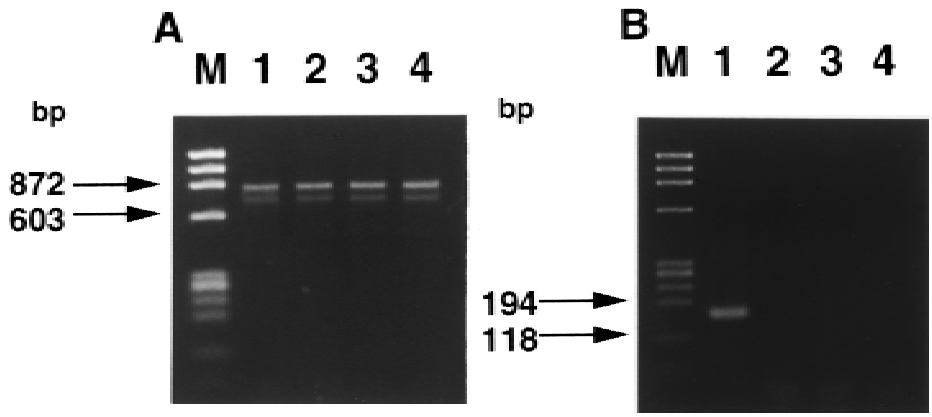

Fig. 3. Longitudinal results in 2 patients. (A) Patient 2 with t(4;11). Lane 1, at diagnosis; Lane 2, 2 months in PR; Lane 3, 4 months in CR; Lane 4, 6 months in 1st relapse. (B) Patient 6 with t(9;11). Lane 1, at diagnosis; Lane 2, 4 months in CR; Lane 3, 5 months in CR; Lane 4, 23 months in CR. A ϕX174 digestion marker is shown at the left.

could detect the chimeric mRNA in the presence of less than 4 ng of total RNA, which represented a final dilution between 10^{-3} and 10^{-4}. The result for patient 6 is shown in Fig. 5.

DISCUSSION

MLL gene on 11q23 is one of the genes most frequently associated with leukemia-specific translocations in childhood [1]. Among more than 10 partner genes, *AF-4/FEL/LTG4* on 4q21, *AF9/LTG9* on 9p22, and *ENL/LTG19* on 19p13 are the most frequently involved [23,24,26,29]. Recent cytogenetic analysis of complex translocation and molecular analysis of these genes have revealed that the critical genetic event in leukemogenesis of 11q23 translocations is the formation of the der(11) chromosome [28,46]. In this study, we performed RT-PCR to detect the resultant der(11) chimeric mRNAs produced from t(4;11), t(9;11), or t(11;19) in 14 leukemia patients with *MLL* gene rearrangement.

Although we set sense primers upstream of the breakpoints to cover the der(11) chimeric mRNA, we could not detect any chimeric mRNA in each patient with t(9;11) and t(11;19). This result suggested that the breakpoints on *AF-9/LTG9* and *ENL/LTG19* gene were located downstream of the antisense primers designed in this study. For instance, a more 3′ exon might have been involved in t(9;11) translocation. Alternatively, other genes 19p13 such as *ELL/MEN* may be involved. Interestingly, *MLL-AF-9/LTG9* fusion gene could be detected in 1 of 4 patients with unknown karyotype. This result clearly showed that RT-PCR is a useful approach for

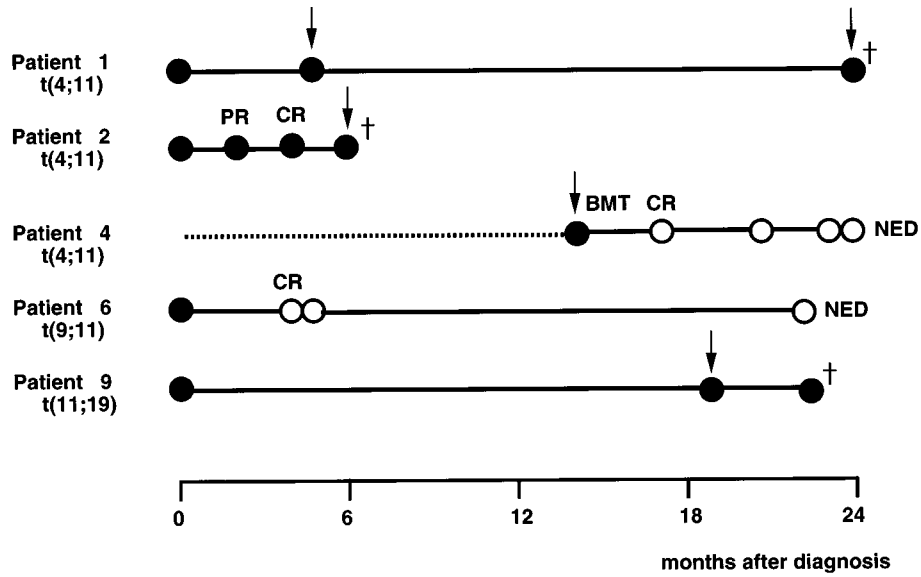

Fig. 4. Longitudinal RT-PCR results in 5 patients. Each circle represents an RT-PCR assay performed at the indicated time: closed circle, positive assay; open circle, negative assay. PR and CR indicates the point at which the patient entered hematological partial and complete remission, respectively. Vertical arrow indicates the time of clinical relapse. BMT denotes bone marrow transplantation. Cross indicates the patient's death. NED (no evident disease) indicates that the patient remains in CR at the most recent follow-up.

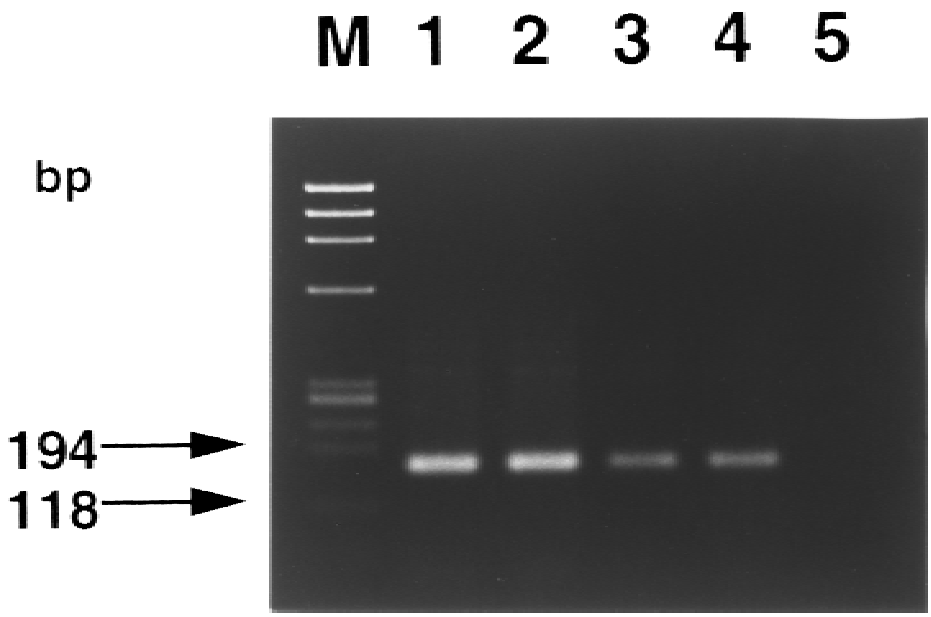

Fig. 5. Sensitivity assay of RT-PCR method applied to leukemic cells of Patient 6. RNA obtained at diagnosis was serially diluted to 10^{-4} by mixing with normal PB, reverse transcribed, and amplified using a pair of primers MLL-EX7 and LTG19-AS1. Lanes 1 through 5 show amplification of RNA dilutions of 1, 10^{-1}, 10^{-2}, 10^{-3}, and 10^{-4}, respectively. A ϕX174 digestion marker is shown on the left.

detecting which partner gene is involved in the translocation.

In this study, we have not sequenced the amplified products. Considering the results of Southern blot analysis, and comparing the size of the amplified products with previously reported ones, all of the chimeric mRNAs detected in this study were estimated to be the same as found so far [26,29,47]. We identified 2 different kinds of products in patients 1, 2, 7, and 9. The difference in size between these 2 products was approximately equal to the size of exon 8, indicating the presence of the alternative splicing which has previously been reported [29,47].

The clinical significance of the detection of MRD in CR patients by RT-PCR remains to be established. In *PML-RAR*α positive acute promyeloid leukemia (APL)

with t(15;17), monitoring of MRD by PCR is extremely useful for the management of the disease [48]. PCR-positivity could be an indicator of relapse, whereas PCR-negativity could be considered a therapeutic goal in APL. In contrast, in cases of *AML1-MTG8*-positive AML(M2) with t(8;21) and *BCR-ABL*-positive chronic myeloid leukemia (CML) with t(9;22), the persistence of minimal residual leukemic cells has been reported in patients during the long CR phase [49,50]. This result suggested that the complete elimination of leukemic cells is not necessarily required for long-term remission. It is widely held that most human cancers result from the accumulation of several genetic changes, including the activating mutations in oncogenes and the loss of function mutations in tumor suppressor genes [51]. It is hypothesized, therefore, that the specific translocations such as t(8;21) and t(9;22) alone are not sufficient; some additional mutational event is necessary for the expression of a clinically aggressive disease. As for leukemias with *MLL* gene rearrangement, it remains unclear whether only the translocation of *MLL* gene is sufficient to express a clinically aggressive state.

So far, it is reported by two groups that patients in whom chimeric mRNA could not be detected after achieving CR remained in CR, and patients in whom chimeric mRNA was continuously detected relapsed and died afterwards [52,53]. We were able to serially study the clinical course of 5 patients. Our data were compatible with these reports. Patients 4 and 6, in whom chimeric mRNA could not be detected after achieving CR, remained in CR. On the contrary, in patient 2, chimeric mRNA could be detected not only at diagnosis and relapse but also in partial remission (PR) and even in CR, and he soon relapsed and died. This case showed that positivity of MRD is a good predictive factor for relapse. These findings suggest that RT-PCR is a useful approach for monitoring MRD in patients with *MLL* gene rearrangement. Long-term and prospective investigation of a larger series of patients is needed to confirm the clinical significance of monitoring MRD by the RT-PCR method.

ACKNOWLEDGMENTS

We thank some members of Tokyo Children's Cancer Study Group, for their gifts of samples and clinical data.

REFERENCES

1. Rabbits TH: Chromosomal translocations in human cancer. Nature 372:143–149, 1994.
2. Raimondi SC, Kalwinski DK, Hayashi Y, Behm FG, Mirro J Jr, Williams DL: Cytogenetics of childhood acute nonlymphocytic leukemia. Cancer Genet Cytogenet 40:13–27, 1989.
3. Raimondi SC: Current status of cytogenetic research in childhood acute lymphoblastic leukemia. Blood 81:2237–2251, 1993.
4. Pui C-H, Raimondi SC, Murphy SB, Riberio RC, Kalwinsky DK, Dahl GV, Crist WM, Williams DL: An analysis of leukemic cell chromosomal features in infants. Blood 69:1289–1293, 1987.
5. Cimino G, Lo Coco F, Biondi A, Elia L, Luciano A, Croce CM, Masera F, Mandelli F, Canaani E: ALL-1 gene at chromosome 11q23 is consistently altered in acute leukemia of early infancy. Blood 82:544–546, 1993.
6. Pedersen-Bjergaard J, Philip P: Balanced translocations involving chromosome bands 11q23 and 21q22 are highly characteristic of myelodysplasia and leukemia following therapy with cytostatic agents targeting at DNA-topoisomerase II. Blood 78:1147–1148, 1991.
7. Larson RA, Le Beau MM, Ratain MJ, Rowley JD: Balanced translocations involving chromosome bands 11q23 and 21q22 in therapy-related leukemia. Blood 79:1892–1893, 1992.
8. Albain KS, Le Beau MM, Ullirsch R, Schumacher H: Implications of prior treatment with drug combinations including inhibitors of topoisomerase II in therapy-related monocytic leukemia with a 9;11 translocation. Genes Chromosomes Cancer 2:53–58, 1990.
9. Pedersen-Bjergaard J, Philip P: Two different classes of therapy-related and de-novo acute myeloid leukemia? Cancer Genet Cytogenet 55:119–124, 1991.
10. Gill Super HJ, McCabe R, Thirman MJ, Larson RA, Le Beau MM, Pederson-Bjergaard J, Philip P, Diaz MO, Rowley JD: Rearrangement of the MLL gene in therapy-related acute myeloid leukemia in patients previously treated with agents targeting DNA-topoisomerase II. Blood 82:3705–3711, 1993.
11. Ziemin-van der Poel S, McCabe NR, Gill HJ, Espinosa R III, Patel Y, Harden A, Rubinelli P, Smith SD, Lebeau MM, Rowley JD, Diaz MO: Identification of a gene, *MLL*, that spans the breakpoint in 11q23 translocations associated with human leukemias. Proc Natl Acad Sci USA 88:10735–10739, 1991.
12. Yamamoto K, Seto M, Akao Y, Iida S, Nakazawa S, Oshimura M, Takahashi T, Ueda R: Gene rearrangement and truncated mRNA in cell lines with 11q23 translocation. Oncogene 8:479–485, 1993.
13. Tkachuk DC, Kohler S, Cleary ML: Involvement of a homolog of *Drosophila trithorax* by 11q23 chromosomal translocations in acute leukemias. Cell 71:691–700, 1992.
14. Cimino G, Moir DT, Canaani O, Williams K, Crist WM, Katzav S, Cannizzaro L, Lange B, Nowell PC, Croce CM, Canaani E: Cloning of *ALL*-1, the locus involved in leukemias with the t(4;11)(q21;q23),t(9;11)(p22;q23), and t(11;19)(q23;p13) chromosome translocations. Cancer Res 51:6712–6714, 1991.
15. Gu Y, Nakamura T, Alder H, Prasad R, Canaani O, Cimino G, Croce CM, Canaani E: The t(4;11) chromosome translocation of human acute leukemias fuses the ALL-1 gene, related to Drosophila trithorax, to the AF-4 gene. Cell 71:701–708, 1992.
16. Djabali M, Selleri L, Parry P, Bower M, Young BD, Evans GA: A trithorax-like gene is interrupted by chromosome 11q23 translocations in acute leukemias. Nature Genet 2:113–118, 1992.
17. Parry P, Djabali M, Bower M, Khristich J, Waterman M, Gibbons B, Young BY, Evans G: Structure and expression of the human trithorax-like gene 1 involved in acute leukemias. Proc Natl Acad Sci USA 90:4738–4742, 1993.
18. Ma Q, Alder H, Nelson KK, Chatterjee D, Gu Y, Nakamura T, Canaani E, Croce CM, Siracusa LD, Buchberg AM: Analysis of the murine all-1 gene reveals conserved domains with human ALL-1 and identifies a motif shared with DNA methyltransferases. Proc Natl Acad Sci USA 90:6350–6354, 1993.
19. Zeleznik-Le NJ, Harden AM, Rowley JD: 11q23 translocations split the ''AT-hook'' cruciform DNA-binding region and the transcriptional repression domain from the activation domain of the mixed-lineage leukemia (MLL) gene. Proc Natl Acad Sci USA 91:10610–10614, 1994.
20. Thirman MJ, Gill HJ, Burnet RC, Mbangkollo D, McCabe NR,

Kobayashi H, Ziemin-van der Poel S, Kaneko Y, Morgan R, Sandberg AA, Chaganti RSK, Larson RA, Le Beau MM, Diaz MO, Rowley JD: Rearrangement of the MLL gene in acute lymphoblastic and acute myeloid leukemias with 11q23 chromosomal translocations. N Engl J Med 329:909–914, 1993.

21. Bernard OA, Mauchauffe M, Mecucci C, Van Den Berghe H, Berger R: A novel gene, AF-1p, fused to HRX in t(1;11)(p32;q23), is not related to AF-4, AF-9 nor ENL. Oncogene 9:1039–1045, 1994.
22. Tse W, Zhu W, Chen HS, Cohen A. A novel gene, AF1q, fused to MLL in t(1;11)(q21;q23), is specifically expressed in leukemic and immature hematopoietic cells. Blood 85:650–656, 1995.
23. Morrissey J, Tkachuk DC, Milatovich A, Francke U, Link M, Cleary ML: A serin/proline-rich protein is fused to HRX in t(4;11) acute leukemias. Blood 81:1124–1131, 1993.
24. Nakamura T, Alder H, Gu Y, Prasad R, Canaani O, Kamada N, Gale RP, Lange B, Crist WM, Nowell PC, Croce CM, Canaani E: Genes on chromosomes 4, 9, and 19 involved in 11q23 abnormalities in acute leukemia share sequence homology and/or common motifs. Proc Natl Acad Sci USA 90:4631–4635, 1993.
25. Prasad R, Gu Y, Alder H, Nakamura T, Canaani O, Saito H, Huebner K, Gale RP, Nowell PC, Kuriyama K, Miyazaki Y, Croce CM, Canaani E: Cloning of the ALL-1 fusion partner, the AF-6 gene, involved in acute myeloid leukemias with t(6;11) chromosome translocation. Cancer Res 53:5624–5628, 1993.
26. Iida S, Seto M, Yamamoto K, Komatsu H, Tojo A, Asano S, Kamada N, Ariyoshi Y, Takahashi T, Ueda R: MLLT3 gene on 9p22 involved in t(9;11) leukemia encodes a serin/prorine rich protein homologous to MLLT1 on 19p13. Oncogene 8:3085–3092, 1993.
27. Chaplin T, Ayton P, Bernard OA, Saha V, Valle VD, Hillion J, Gregorini A, Lillington D, Berger R, Young BD: A novel class of zinc finger/leucine zipper genes identified from the molecular cloning of the t(10;11) translocation in acute leukemia. Blood 85:1435–1441, 1995.
28. Prasad R, Leshkowitz D, Gu Y, Alder H, Nakamura T, Saito H, Huebner K, Berger R, Croce CM, Canaani E: Leucine-zipper dimerization motif encoded by AF17 gene fused to ALL-1 (MLL) in acute leukemia. Proc Natl Acad Sci USA 91:8107–8111, 1994.
29. Yamamoto K, Seto M, Komatsu H, Iida S, Akao Y, Kojima S, Kodera Y, Nakazawa S, Ariyoshi Y, Takahashi T, Ueda R: Two distinct portions of LTG19/ENL are involved in t(11;19) leukemia. Oncogene 8:2617–2625, 1993.
30. Thirman MJ, Levitan DA, Kobayashi H, Simon MC: Cloning of ELL, a gene that fuses to MLL in a t(11;19)(q23;p13.1) in acute myeloid leukemia. Proc Natl Acad Sci USA 91:12110–12114, 1994.
31. Mitani K, Kanda Y, Ogawa S, Tanaka T, Inazawa J, Yazaki Y, Hirai H: Cloning of several species of MLL/MEN chimeric cDNAs in myeloid leukemia with t(11;19)(q23;p13.1) translocation. Blood 85:2017–2024, 1995.
32. Parry P, Wei Y, Evans G: Cloning and characterization of the t(X;11) breakpoint from a leukemic cell line identify a new member of the forkhead gene family. Genes Chromosomes Cancer 11:79–84, 1994.
33. Schichman SA, Caligiuri MA, Gu Y, Strout MP, Canaani E, Bloomfield CD, Croce CM: *ALL*-1 partial duplication in acute leukemia. Proc Natl Acad Sci USA 91:6236–6239, 1994.
34. Schichman SA, Caligiuri MA, Strout MP, Carter SL, Gu Y, Canaani E, Bloomfield CD, Croce CM: ALL-1 tandem duplication in acute myeloid leukemia with a normal karyotype involves homologous recombination between Alu elements. Cancer Res 54: 4277–4280, 1994.
35. Hayashi Y, Sugita K, Nakazawa S, Abe T, Kojima S, Inaba T: Karyotypic patterns in acute mixed lineage leukemia. Leukemia 4:121–126, 1990.
36. Rubnitz JE, Link MP, Shuster JJ, Carroll AJ, Hakami N, Frankel LS, Pullen DJ, Cleary ML: Frequency and prognostic significance of HRX rearrangements in infant acute lymphoblastic leukemia: a pediatric oncology group study. Blood 84:570–573, 1994.
37. Heerema NA, Arthur DC, Sather H, Albo V, Feusner J, Lange BJ, Steinherz PG, Zeltzer P, Hammond D, Reaman GH: Cytogenetic features of infants less than 12 months of age at diagnosis of acute lymphoblastic leukemia: Impact of the 11q23 breakpoint on outcome: A report of the children's cancer group. Blood 83:2274–2284, 1994.
38. Tsuchida M, Akatsuka J, Bessho F, Chihara H, Hayashi Y, Hoshi Y, Hosoya R, Furukawa T, Ikuta K, Inana I, Ishikawa A, Ishimoto K, Ito K, Kaneko M, Kaneko T, Kato S, Komiyama J, Matsuyama S, Nagao T, Nakazawa S, Nishihara K, Ohira M, Okimoto Y, Ohkawa Y, Ohtsuki H, Sato T, Shibuya A, Shitara T, Sugita K, Taguchi N, Torigoe K, Tsukada M, Tsukimoto I, Tsunematsu Y, Wada E, Yamada K, Yamada K, Yamamoto K, Yamamoto M, Yata J, Nishimura K, Saito T: Treatment of acute lymphoblastic leukemia in Tokyo Children's Cancer Study Group preliminary results of L84–11 protocol. Acta Paediatr Jpn 33:522–532, 1991.
39. Bessho F, Kigasawa H, Tsuchida M, Tsukimoto I, Nakazawa S, Yamamoto M, Tsunematsu Y, Yamada K, Sugita K, Ohkawa Y, Kaneko T, Kaku H, Inana I, Wada E, Kanako M, Itoh K, Shidara T: Improved prognosis of acute nonlymphocytic leukemia in children: Results of the 12th-ANLL protocol of Tokyo Children's Cancer Study Group. Acta Paediatr Jpn 33:533–539, 1991.
40. Bennett JM, Catovsky D, Daniel M-T, Flandrin G, Galton DAG, Gralnick HR, Sultan C: Proposal for the classification of the acute leukemias. French-American-British (FAB) Cooperative Group. Br J Haematol 33:451–458, 1976.
41. Hayashi Y, Eguchi M, Sugita K, Nakazawa S, Hanada R, Yamamoto K, Sato T, Kojima S: Cytogenetic findings and clinical features in acute leukemia and transient myeloproliferative disorder in Down's syndrome. Blood 72:15–23, 1988.
42. Kikuchi A, Hayashi Y, Kobayashi S, Hanada R, Moriwaki K, Yamamoto K, Fujimoto J, Kaneko Y, Yamamori S: Clinical significance of TAL1 gene alteration in childhood T-cell acute lymphoblastic leukemia and lymphoma. Leukemia 7:933–938, 1993.
43. Kawamura M, Kikuchi A, Kobayashi S, Hanada R, Yamamoto K, Horibe K, Shikano T, Ueda K, Hayashi K, Sekiya T, Hayashi Y: Mutations of the p53 and ras genes in childhood t(1;19)-acute lymphoblastic leukemia. Blood 9:2546–2552, 1995.
44. Chomczynski P, Sacchi N: Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. Anal Biochem 162:156–159, 1987.
45. Kaneko Y, Maseki N, Takahashi N, Sakurai M, Hayashi Y, Nakazawa S, Mori T, Sakurai M, Takeda T, Shikani T, Hiyoshi Y: Clinical and hematological characteristics in acute leukemia with 11q23 translocations. Blood 67:484–491, 1987.
46. Rowley JD: The der(11) chromosome contains the critical breakpoint junction in the 4;11, 9;11, and 11;19 translocations in acute leukemia. Genes Chromosomes Cancer 5:264–266, 1992.
47. Yamamoto K, Seto M, Iida S, Komatsu H, Kamada N, Kojima S, Kodera Y, Nakazawa S, Saito H, Takahashi T, Ueda R: A reverse transcriptase-polymerase chain reaction detects heterogenous chimeric mRNAs in leukemias with 11q23 abnormalities. Blood 83: 2912–2921, 1994.
48. Diverio D, Pandolfi PP, Biondi A, Avvisati G, Petti MC, Mandelli F, Pelicci PG, Lo Coco F: Absence of reverse transcription-polymerase chain reaction detectable residual disease in patients with acute promyelocytic leukemia in long-term remission. Blood 82:3556–3559, 1993.

49. Nucifora G, Larson RA, Rowley JD: Persistence of the 8;21 translocation in patients with acute myeloid leukemia type M2 in long term remission. Blood 82:712–715, 1993.
50. Miyamura K, Tahara T, Tanimoto M, Morishita Y, Kawashima K, Morishita Y, Saito H, Tsuzuki S, Takeyama K, Kodera Y, Matsuyama K, Hirabayashi N, Yamada H, Naito K, Imai K, Sakamaki H, Arai O, Mizutani S: Long persistent bcr-abl positive transcript detected by polymerase chain reaction after marrow transplant for chronic myelogenous leukemia without clinical relapse: A study of 64 patients. Blood 81:1089–1093, 1993.
51. Cline MJ: The molecular basis of leukemia. N Eng J Med 330: 328–336, 1994.
52. Biondi A, Rambaldi A, Rossi V, Elia L, Caslini C, Basso G, Battista R, Barbui T, Mandelli F, Masera G, Croce C, Canaani E, Cimino G: Detection of ALL-1/AF4 fusion transcript by reverse transcription-polymerase chain reaction for diagnosis and monitoring of acute leukemias with t(4;11) translocation. Blood 82: 2943–2947, 1993.
53. Janssen JWG, Ludwig W-D, Borkhardt A, Spadinger U, Rieder H, Fonatsch C, Hossfeld DK, Harbott J, Shultz AS, Repp R, Sykora K-W, Hoelzer D, Bartram CR: Pre-Pre-B acute lymphoblastic leukemia: high frequency of alternatively spliced ALL1-AF4 transcripts and absence of minimal residual disease during complete remission. Blood 84:3835–3842, 1994.